PRUNING EFFECTS ON SAP FLOW AND SUGAR PHLOEM UNLOADING IN CURRENT-YEAR SHOOTS OF DIFFERENT AGED GRAPEVINE SHOOTS

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Abstract

The study aimed at providing an efficient and sustainable pruning technique and theory for high quality and stable yield in grape production, sap flow rate in current-year shoots on different aged grapevines, photosynthetic rate of leaves, pruning-induced tylose development in vessel, and the sugar phloem unloading in grape berries. It was found that the sap flow rate changed rapidly with the increase of the water transport distance and changed dramatically with the increasing age of the shoots. The photosynthetic rate and the sugar phloem unloading trend in all treatments were basically remained the same. As the distance of water transportation increased, the sugar phloem unloading in grape berries on the older shoots was significantly lower than that on the younger ones. Tyloses were found in the vessel after pruning, and the vessel partly or completely blocked by tyloses at the wound would continue to increase over time. The results showed that the changes of sap flow rate, photosynthetic rate and sugar phloem unloading were not simultaneous. With the extension of the perennial part, the resistance of water transport became greater; the amount of sugar phloem unloading in berries also decreased with the extension of the perennial part. The study proposed the number of cuts and the length of perennial parts should be minimized without affecting the quality, stability, and sustainability of grape production.

Key words: Pruning, Sap flow rate, Sugar phloem unloading, Tylose, Water transport distance.

Introduction

As an important index for grape berry quality, sugar content is closely related to the transportation and allocation of photo-assimilates in grapes. The distribution of photo-assimilates is an important strategy for plants to adapt to various environmental constraints (Bidell et al., 2000). Many studies have been conducted on the impact of environmental factors such as light, temperature, soil, water and nutrients on the distribution of photo-assimilates (Yokotsuka et al., 1999; Bergqvist et al., 2001; Rance et al., 2009; Spayd et al., 2002; Ristic et al., 2007; Cohen et al., 2008; Lim et al., 2010). These environmental factors affect the allocation of photo-assimilates in plants by the form of production, transportation, absorption, and utilization of the assimilates. The distribution of photo-assimilates between source libraries is mainly subjected to two aspects. One is the concentration gradient between source libraries and the other is the resistance between source libraries (Bancal & Soltani, 2002; Wang & Huang, 2003).

Water is an important factor for grape growth and development. It participates in and affects the material circulation and energy flow of the vineyard ecosystem. At the same time, the movement and circulation of moisture are also affected by the vineyard ecosystem. The transportation status of grape water is closely related to the condition of xylem. Variations in the content of synthetic cellulose and α-cellulose in different parts of xylem of the grape affect the transport performance of the various parts of the water. The hydroxyl group in α-cellulose is hydrophilic, and the α-cellulose content between the xylem sections is higher than the nodes. When the water is transported to the junction area, the sap flow rate naturally decreases due to the reduced affinity (Sun & Shen, 2008). Moreover, the cells that consist the node are uneven, there are more small vessels, the ducts are detour around, and the degree of differentiation of the node cells is also uneven. Some cells are still in the differentiation stage, the protoplasm is thick, and the orientation of the cells is irregular and increases the unit distance. While the length, the retention time of water in the junction area is increased, and the sap flow rate decreases (Schulte & Brooks, 2003; Gullo et al., 2004).

Pruning at different locations along the shoot is a normal part of viticulture, but it may lead to wedge-shaped organization and tylose formation in vessels near cuts (Sun et al., 2007; Zhao et al., 2013). These physical damages can profoundly affect the transportation capacity of vessel and sieve tube (Zhao et al., 2014). As an important part of water transport and exchange with the environment, the xylem of grape is closely related to the transduction and transpiration of grape water, and also reflects the influence of environmental factors on plant growth and development. The water potential, leaf area and transpiration of the leaves gradually decrease with the extension of water transportation distance (Esau, 1977). When photo-assimilates is transported in the phloem, there must be the participation of the water in the xylem, and the phloem that is separated from the xylem has only the diffusion migration of photo-assimilates and water, and photo-assimilates can not be transported alone (Liu et al., 1998). The congeners are transported in the phloem in the form of liquid flow, and the water in the liquid flow mainly comes from the xylem. With the extension of the water transportation distance, the photosynthetic capacity of the shoots gradually decreases due to the increasing of the leaves and the limitation of stoma, and with the extension of the water transportation distance, the water transportation is affected by the structure of the vessel and the resistance increases (Schulte & Brooks, 2003; Gullo et
Changes in the sap flow rate of current-year shoots on 2-year-old and perennial shoot: From July 20, 2011, current-year shoots with basically the same growth and development status were selected and the sap flow rates of the first and fifth shoots on the shoot-A were determined, labeled A1 and A2 respectively. The sap flow rates of the first and fifth shoots on the shoot-B were measured and labeled B1 and B2, respectively. Repeated 3 times for five days’ continuous determination.

The soil within 0.5 m around the above experimental grapevines were covered with thin films to prevent water loss through the surface transpiration. Other vineyard managements were according to the normal vineyards managements. The tested shoots were gently polished and smoothed with sand cloth before measurement, and the diameter of the measured site was accurately determined. G4 grease was then applied to the polished installation area. We measured the sap flow rate of the current-year shoots using the Dynagage system (flow 32-1k, Dynamax, Inc., Houston, TX, USA) (Schepper et al., 2010). After the equipment stabilized, measurements lasted continuously for 3 days, the data was recorded automatically once every 30 min.

Differences of leaves’ photosynthetic rate of current-year shoots on perennial shoots: At the same time with measuring the sap flow rate of current-year shoots, selecting the sunny day from 8: 00 am to 19: 00 pm by using Li-6400 photometer (LI-COR Co. Ltd., America), the photosynthesis rate of the leaves in the middle of the first and fifth shoots on the shoot-A and shoot-B were measured every 1 h, and used an open-circuit system with natural light intensity. For each treatment three grapevines were measured, and the results were expressed as the mean of three measurements.

Measurements of sugar phloem unloading in grape berries of current-year shoots: While determining the sap flow rate and photosynthetic rate, sugar phloem unloading in grape berries was tested by New Berry-cup Technology at veraison stage (Wang et al., 2003). On the selected current-year shoots (A1, A2, B1 and B2) of every grapevine, one “berry-cup” experiment system was established and replicated three times. The sugar unloading sap was collected once every 60 min (the buffer solution in the berry cup was saturated after 60 min), the bleeder valve was opened during collection, and the collected buffer solutions were transferred into tubes for freezing. Thereafter, the bleeder valve was closed, and new buffer solution was injected into the empty cups with injectors. The empty cups were changed once every day. In order to accurately reveal the sugar unloading of grape fruits, sugar content measurement was not carried on the first unloading sap (7: 00 am). Instead, the measurements started with the second collecting cup, and the collecting of unloading sap lasted from 8: 00 am to 7: 00 pm. There were 12 collections for each experimental system every day and an average of three replicates was derived. The collected unloading sap was stored at -70°C. Samples were dried in the freeze dryer, and diluted to the same volume with distilled water. High pressure liquid chromatography (HPLC) (Wang, 2004) was utilized for measuring total sugar (glucose), and the result was derived from the average of three measurements.
Observation of tylose generation and development: After harvesting, 60 grapevines were selected. The fifth current-year shoots were cut above the shoot-A and shoot-B, pruning surface was 5 cm from the shoots. A set of two samples (2 cm length) was taken from the apical side of each pruning surface every day, the sampling lasted for continuous 10 days. They were placed in glutaraldehyde solution and FAA respectively for the preparation of paraffin slices and transmission electron microscope sample to observe.

Preparation and observation of paraffin sections: Grape skins were cut into 3-mm slims in the stationary liquid. FAA-fixed samples were washed in tap water for 48 h, and then softened for 7 d in a 80°C water bath containing a mixture of polyethylene glycol (PEG) and acetic acid ($M_{\text{PEG}} : M_{\text{AceticAcid}} = 1:1$). Samples were made by employing the conventional paraffin sectioning method and sliced with a rotary microtome. Samples were dyed with sarranine-fast green, and an OLYMPUS CK2 microscopic camera (CK2; Olympus, Tokyo, Japan) was used for imaging.

Preparation and observation of transmission electron microscope: After placing the sample into the glutaraldehyde for 4 h, cutting of the sample vertically and horizontally was done with a sharp blade into strips about 2 mm length, matchstick thickness, and evacuating for 30 min. and were rinsed with 0.1 M phosphate buffer (pH 6.8) 4 times, 15-20 min each. The samples were placed into osmic acid (4°C) for 2 h, to rinse with 0.1 M phosphate buffer (pH 6.8) 5 times, 15-20 min each and were then dehydrated 30 min and 3 times in 30%, 50%, 70%, 80%, 90%, and 100% acetone, respectively buried with Epon812 packs, and sectioned to 60-90 mm thickness by using ultramicrotome, and finally observed and photographed under transmission electron microscope.

All vessels in each area were categorized as vessels without tyloses, vessels partially filled with tyloses, or vessel completely occluded with tyloses (the vessel was padded 80% of the cross-sectional area by tyloses). Two parameters, percentage of vessels with tyloses and percentage of vessels occluded completely by tyloses, were calculated to quantify tylose development. The grape skins were cut into 3-mm slims in the stationary liquid to make a transmission electron microscope sample, and the sugar phloem was observed unloading in the grape skin.

Results

Changes in the sap flow rate of current-year shoots on perennial shoot: As seen in Fig. 2, the changing trends of sap flow rate of current-year shoots on different aged shoots were consistent. The starting time of the sap flow of each shoot was around 6: 00 A.M., and enter a rapid increase period was at about 8: 00 A.M. The sap flow rate of the current-year shoots A1 and B1 showed a typical bimodal curve, while the sap flow rate of A2 and B2 showed a typical unimodal curve.

The sap flow rates of A1 and B1 reached the first peak between 11: 00 AM and 12: 00 PM and entered the valley bottom from 13: 00 PM to 14: 00 PM, and the sap flow rate rose rapidly reaching the second peak at about 15: 00 PM, and then rapidly declined until about 20: 00 PM to reach a stabilization period close to 0. Sap flow rate of A2 and B2 reached the peak values at about 12: 00 PM and remained until 15: 00 PM, and then entered a rapid reduction period until the stabilization period close to 0 at about 20:00 PM. It was found that the sap flow rate of A1 and B1 changed slowly compared with that of A2 and B2. On the shoot-B with a longer perennial part, the sap flow rates of B1 and B2 varied greatly with the extension of the water transport distance, while the difference between A1 and A2 was relatively small, while greater than that of A2 and B2.

Differences of leaves’ photosynthetic rate of current-year shoots on perennial shoots: Fig. 3 showed that the daily changes of photosynthetic rate of each shoot leaf showed a bimodal curve, and the first peak appeared at about 11: 30 AM, which was the highest peak of photosynthetic rate throughout the day (Fig. 3). The second peaks were smaller than the first peaks and appeared at about 15: 00 pm. Due to the photosynthetic midday-depress, the photosynthetic rate of each shoot entered a low point at about 14: 00 PM. The photosynthetic rates of A1, A2 and B1 were not obvious, and the photosynthetic rate of B2 was the minimal one.

Sugar phloem unloading in grape berries of current-year shoots on different aged shoots: The trends of sugar phloem unloading in grape berries of current-year shoots on different aged shoots were basically the same, showing a bimodal curve. The amount of sugar phloem unloading began to increase from 8: 00 AM while reaching a peak at about 11: 00 AM, and then gradually declined until entering a “low trough” at about 14: 00 PM. The slow recovery began at 15: 00 PM with a second peak at about 18: 00 PM (Fig. 4) and then gradually decreased, with the second peak significantly lower than the first peak. Sugar phloem unloading of B2 was significantly lower than that of A1, A2 and B1 ($p \leq 0.05$). Sugar phloem unloading of A2 also tended to decrease (Figs. 4 and 5), but the difference between A1 and B1 was not significant ($p \geq 0.05$). The values of A1 and B1 were the largest, and there was no significant differences between them ($p \geq 0.05$).

Generation and development of tylose: Tyloses developed in response to prune from both axial parenchyma cells and ray parenchyma cells. Typically, there were no tyloses (Figs. 6a, 6b) in vessels of the shoots used in the experiment except the shoots were wounded by pruning (Fig. 6c). Once the pit membrane and its outside covertures were broken, the bilayer protuberance became the vessel element to form tyloses. Tyloses might start to break in the xylem vessels when the axial wood parenchyma cells and wood ray parenchyma became visible. The results showed that small round to oval tyloses in some vessels were observed within 1 day after pruning (Fig. 6c). If the cell nucleus and partial cytoplasm of the parenchyma cell were accumulated in the vessel, the formed tylose could divide (Fig. 2d) and eventually blocked the whole vessel lumen (Fig. 2e). Interestingly, we found the evidences of wound gels in some section of current year shoots (Figs. 2g, 2h).
Discussion

Relationship between photosynthetic rate, sap flow rate and sugar phloem unloading: At 8:00 AM, the light intensity was weak, the sap flow rate and the photosynthetic rate of the leaves were low (Figs. 2 and 3). As the intensity of illumination increased, the concentration of CO₂ in the cell gap decreased, the temperature increased, and the photosynthesis and transpiration were accelerated. Photosynthetic rate and sap flow rate of shoots rose rapidly. The first peak in photosynthetic rate occurred about 10:00 AM in the morning (Fig. 3) with the sugar phloem unloading of grape berries occurring the first peak at about 11:00 AM (Fig. 4). However, as the intensity of illumination continued to increase, the temperature continued to rise, the high temperature threat forced the grape berry to reduce its ability to compete with photosynthetic products, the storage effect of grape berries decreased, and the amount of sugar phloem unloading decreased (Fig. 4). With the increasing of temperature, the sap flow rate reached its first peak between 12:00 PM and 13:00 PM (Fig. 2). At this time, the water content of the leaves and the concentration of CO₂ in the cell space continued to decrease, the water deficit in the leaves caused partial stomata closure, the activity of rubisco was reduced occurring the photosynthetic midday-depress (Bertamini et al., 2002; Wu et al., 2005), photosynthetic rate (Fig. 3) and sap flow rate of A₁ and B₁ (Fig. 2) entered a trough at about 14:00 pm. Due to the closure of some stomata in the leaves, the transpiration rate decreased, the water content of the leaves increased, and the photosynthetic rate began to increase rapidly again, reaching a second peak at about 15:00 pm.

Photosynthetic rate decreased as solar radiation continued to decrease (Fig. 3). As the temperature gradually decreased, the porosity of the leaves increased, the sap flow rate increased rapidly, and the second peak occurred at about 15:00 pm (Fig. 2). As the intensity of illumination decreasing, the temperature of the leaves gradually decreased, and the sap flow rate decreased rapidly. At about 20:00 pm, it reached a stabilization period that tended to 0 until it entered a rapid increase at 7:00 am the next morning when the temperature continued to increase. After 15:00 pm, the temperature of the leaves was still high, the respiration and photorespiration of the leaves were strong, and the amount of sugar phloem unloading in berries increased slowly until about 18:00 pm. The respiration of the leaves was weak, and the photosynthetic assimilates was mainly transferred to the grape berries appearing the second peak. As the intensity of light weakened, photosynthesis continued to weaken (Kemaniana et al., 2005). The unloading of grape fruit sugar gradually decreased and re-entered the trough at 17:00 pm (Fig. 4).

Observation of the tylose generation and development of current-year shoots on different aged shoots: After first day pruning, there would be tylose in the vessel, and over time, the vessel whether partly or completely blocked by tyloses at the shear would continue to increase. Finally, the shoots on the perennial branches would reach 80% and 35%, and the shoots on two-year shoots reached 65% and 30% respectively, which can profoundly affect the patterns of water movement within the developing xylem networks (Figs. 7 and 8).

Fig. 2. The diurnal time course of sap flow for different current-year shoots of Guyot training system. A₁ and A₂: Sap flow rate of the first (A₁) and fifth (A₂) current-year shoot of shoot-A; B₁ and B₂: Sap flow rate of the first (B₁) and fifth (B₂) current-year shoot of shoot-B.

Fig. 3. Diurnal variations of photosynthetic rates of different current-year shoots under Guyot training system. A₁ and A₂: Photosynthetic rate of the first (A₁) and fifth (A₂) current-year shoot of shoot-A; B₁ and B₂: Photosynthetic rate of the first (B₁) and fifth (B₂) current-year shoot of shoot-B.

Fig. 4. Diurnal buffer sugar content of berry-cup on different fruiting current-year shoot as a function of sampling time. The buffer solution of the berry-cup becomes saturated after 60 min. The results shown are the mean values (n=3 replicate berry-cups) of sugar efflux (mg/mL) from the peeled berry into the buffer solution. A₁ and A₂: Sugar unloading rate of the berry-cup on the first (A₁) and fifth (A₂) current-year shoot of shoot-A; B₁ and B₂: Sugar unloading rate of the berry-cup on the first (B₁) and fifth (B₂) current-year shoot of shoot-B.
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Fig. 5. Sugar content of different fruiting current-year shoot in grape skin (600x). A: Fruit in shoot 1 of shoot-A; B: Fruit in shoot 1 of shoot-B; C: Fruit in shoot 5 of shoot-A; D: Fruit in shoot 5 of shoot-B.

Changes in the sap flow rate of current-year shoots on perennial shoot: The vessel is the subject of water transport, but most plant vessels do not extend in parallel, making each vessel in contact with multiple vessels. With the extension of the water transport distance, the vessel will deviate from the longitudinal direction and cause the water transport to be affected by transverse resistance (Kitin et al., 2004). Moreover, lateral transportation of water will occur, and the resistance to lateral transportation of water will be greater (Taneda & Tateno, 2007; Halis, 2012). During the upward transport of water, a series of incompletely differentiated vessels will be passed, which will increase the resistance to water transport (Meuser & Frensch, 1998) and reduce the sap flow rate. Pruning is an important management measure in the management of grape cultivation, but tylose of the vessel after pruning will even completely block part of the vessel, which will block the water transport of the vessel like an undifferentiated complete vessel. The more perennial parts of the vine, the more cuts on the tree body, the greater the amount of clogging of the vessel by tylose, and the more obvious the impact on water transport (Zhao et al., 2014; Luan et al., 2018).
Grape is a segmented vine plant, and the node is the main body of grape water transportation. The alpha-cellulose content of the internode region of the grape xylem is higher than that of the node area, making the water contact angle of the internode vessel smaller than that of the node vessel, and the internode has higher transport and adsorption performance (Sun & Shen, 2008). The cross section in the middle of the vessel is significantly larger than the perforated cross section between the vessel joints, and the terminal aperture between the tube cells is smaller than the central diameter, so that the friction and resistance when the water is transported from one node to the other increases, affecting the sap flow rate (Shang, 2011). Moreover, the cell size of the constituent node is different, the vessel is curved, the vessel diameter is small, the node cell differentiation is low, the protoplasm is thick, the cell arrangement is irregular, and the water distance and resistance are increased (Paul et al., 2003; Lo et al., 2004; Schulte & Brooks, 2003). The longer the perennial part of the grape, the more segments existed, the lower the sap flow rate showed.

The lengths of the perennial part of the water transport on the shoots A1 and B1 were the same, and the sap flow rate were basically the same. Although the water was transported to A2 and B2 at the same distance, the water transport to B2 took place after a period of perennial part (shoot-B), and the liquid flow rate was reduced due to the influence of the pruning-induced tylose, so the sap flow rate of A2 was greater than that of B2. Although the transport of moisture to A2 was consistent with the perennial parts transported to A1 and B1, there was no effect on the penetration on A2, but the distance of water transport was extended, and the resistance of undifferentiated ducts and spiraling small ducts increased, sap flow rate of A2 was less than that of A1 and B1 (Fig. 2).

Change of sugar phloem unloading in grape berries of current-year shoots on different aged shoots: The transportation of higher plant optical contractual compounds is greatly affected by the development of vascular bundles, and the transmission status of optical contractual compounds from source to sink is affected by the size, quantity, interconnection and circulation ability of sieve tubes. However, there were also studies (Wardellaw, 1990) that reported that the vascular bundle had a strong transport capacity (except for the apex meristem tissue) and usually did not affect the transport distribution of congeners.

The distribution of congeners is affected by the transport distance of congeners, but it is generally believed that the length of vascular bundles has little effect on the distribution of congeners (Cook & Evans, 1983; Marcelis, 1996). Even the resistance of vascular bundles does not affect the distribution of congeners under the condition that the congeners are adequately supplied (Minchin et al., 1993). However, with the extension of the water transport distance, the water transport is affected by the structure of the vessel and the resistance increases (Schulte and Brooks, 2003; Gullo et al., 2004). The development of grape berry is relatively delayed, the ability of grape berries to storage photosynthetic assimilates from the leaves gradually decreased, and the sugar phloem unloading gradually decreases (Farrar & Jones, 2000).

The sap flow rate of the fifth tip (A2) on shoot-A was not significantly different from that of the first tip (A1 and B1, respectively) on the shoot-A and shoot-B, and the development status of the grape berries was basically the same. The difference in sugar discharge was not significant. The sap flow rate of the 5th tip (A2) on the shoot-A was greater than that of the 5th tip (B2) on the shoot-B. The fruit develops rapidly, the ability of berries to obtain photosynthetic assimilates was strong. Therefore, the amount of sugar phloem unloading was also large (Fig. 4).

Change the generation and development of tylose: In cultivated grapevines, pruning at different locations along the shoot is a normal management of viticulture, but it may lead to wedge-shaped organization and tylose formation in vessels near cuts. These physical damages can profoundly affect the transportation capacity of vessel and sieve tube. Reasonable pruning can improve the adaptability of grapes and the environment and grape quality.
However, tylose can also be found in some unwounded vessels that lose water transport capacity, the presence of tylose is also observed in the old leaves (Parameswaran et al., 1985; Ranjani & Krishnamurthy, 1988). After pruning, researchers such as Sun et al., (2008; 2013) found that the largest amount of tyloses in the vessels near the grape medulla were mainly due to the weakening of the water transport function near the medulla.

Water transport function of the vessel in the perennial shoot is reduced, so tylose is more likely to form in the vessel cavity (Zhao et al., 2013, 2014; Luan et al., 2018). In this experiment, although there was less tylose in the perennial shoot (shoot-B) in first few days after pruning, the vessel whether partly or completely blocked by tyloses at the shear would continue to increase than that of shoot-A (Figs. 7 and 8).

Conclusion

During the fruit development, the change of sugar phloem unloading in berries showed a bimodal curve, the change of photosynthetic rate obviously presented the photosynthetic midday-depress, but the changes between sap flow rate, photosynthetic rate and sugar phloem unloading were not synchronous. With the increase distances of the perennial parts of grapes, the resistance of water transport was greater. In addition, the amount of tylose induced by pruning perennial vines and the amount of completely blocked vessels by tylose also increased. The greater the resistance of water transport, the lower the amount of sugar phloem unloading with the extension of the perennial part. The development of tyloses depended on the pruning location and post-pruning time. Tylose was few in the secondary xylem of unwounded shoots. The formation of tyloses and gel was not related to the width and length of the vessel, while the number of tylose was significantly positively correlated with the quantity of parenchyma cells of the secondary xylem. The study concluded that the number of cuts and the length of perennial parts should be minimized without affecting the quality, stability, and sustainability of grape production.

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